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The aim of the study is to elucidate the role of human selenium binding protein (hSP56) in selenium induced cell apoptosis in cancer cells. During the second year of the project we demonstrated that the human prostate (PC-3) cells overexpressing hSP56 protein show changed growth characteristic and increased sensitivity to selenium compounds. To understand the biological role(s) of hSP56, a yeast two-hybrid screening has been performed to identify the proteins interacting with hSP56 from human prostate cDNA library. The screening identified two potential proteins as interacting partners of hSP56, Ubc12 and VDU1. Both proteins are involved in ubiquitination-mediated protein degradation pathway. We hypothesize that hSP56 regulates the protein degradation pathway by altering the properties of Ubc12 and/or VDU1.

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Annual Summary

The aim of the study is to identify the role of human selenium binding protein (hSP56) in selenium induced cell death in human prostate cancer. Previously, our laboratory identified hSP56 as one of the proteins expressed in the slow-growing human prostate cancer cell line, LNCaP, but not in the fast-growing human prostate cancer cell line, PC-3. During the first year of the project two types of cancer cell lines with modified hSP56 expression have been prepared. Anti-sense RNA mediated method was used to suppress the of endogenous hSP56 gene expression in LNCaP cells. The mammalian expression plasmid encoding hSP56 gene has been used to obtain PC-3 cells with inducible expression of exogenous hSP56 protein. Additionally, metabolic labeling followed by immunoprecipitation demonstrated that hSP56 contains covalent selenium, indicating that the hSP56 protein interacts with selenium in a different way from other types of selenium-containing proteins (e.g. glutathione peroxidase and selenoprotein P) that have selenium incorporated into the polypeptide as selenocysteine.

In the second year of the training the effect of up-regulation of hSP56 in the prostate cancer cell line PC-3 with the inducible expression of exogenous hSP56 protein were studied. When induced by tetracycline, this cell line is capable of expressing many folds higher level of hSP56 protein. The overexpression of hSP56 in PC-3 cells has changed cell growth characteristic and made the cell line more sensitive to selenium compounds like known for the LNCaP cells (manuscript in preparation). This indicates that hSP56 may be a key molecule responsible for the selenium mediated cell growth blockade observed in the slow-growing, androgen-responsive human prostate cancer cell line, LNCaP cells.

However, little is known about hSP56's mechanism. Despite the implication of hSP56 in selenium-mediated cell cycle control, its functions and signaling pathways have not been characterized. Pattern and motif searches with the deduced amino acid sequences of hSP56 revealed no important functional sites or signatures. We hypothesized that hSP56 functions through protein-protein interactions.

Therefore, the experiments to identify proteins interacting with hSP56 using yeast two-hybrid screening were carried out. We used the full-length hSP56 as bait and screened over 4×10^6 colonies obtained from the human prostate cDNA library. The C-terminal 172 amino acids of pVHL-interacting deubiquitinating enzyme (VDU1) and the full-length NEDD8 conjugating enzyme (Ubc12) were identified as potential interacting protein partners of hSP56. For both Ubc12 and VDU1 fragment, the activation of the reporter genes was observed only with the hSP56 containing bait plasmid and not with the control bait plasmid. This experiment confirmed the specificity of the interactions in the yeast two-hybrid system.

Ubc12 and VDU1 proteins are involved in ubiquitin-mediated protein degradation pathways, suggesting that hSP56 also takes important part in regulating the protein degeneration. Based on these findings, several addition experiments are being performed to furtherer characterize the interactions between hSP56 and Ubc12 or VDU1 and to explore the biological significance of the interactions. All proteins were expressed and purified from bacteria and we are confirming the interactions by *in vitro* binding assay. I*n vivo* interactions are also being examined by co-immunoprecipitation experiment. The effect of selenium compounds on the interactions and the biological significance of the interactions will also be explored.

Key accomplishment

- Stably transfected PC-3 cells overexpressing hSP56 protein showed a changed growth characteristic and increased sensitivity to selenium compounds.

- This indicates that hSP56 may be a key molecule responsible for the selenium mediated cell growth blockade observed in LNCaP cells.

- The yeast two-hybrid system has identified several potential hSP56 interacting partners, including pVHL-interacting deubiquitinating enzyme (VDU1) and NEDD8 conjugating enzyme (Ubc12).

- Both VDU1 and Ubc12 are involved in ubiquitination-mediated protein degradation indicating the role of hSP56 in this process.